HIGH RESOLUTION IMAGING OF MALARIA PARASITES: LIGHT, X-RAYS OR ELECTRONS?

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ABSTRACT: Efforts to answer the major medical and biotechnology questions of the 21st century will be heavily reliant on the use of advanced imaging techniques. Indeed, new microscopy techniques are already providing amazing insights into the cellular landscape. We have used high resolution imaging methods to explore the sub-cellular topography of the malaria parasite, *Plasmodium falciparum*.

The resolution of conventional optical microscopy is limited to about half the wavelength of the illuminating photons. Now, new super-resolution techniques, such as 3-D structured illumination microscopy (SIM), are providing information beyond the diffraction limit. 3D-SIM can be used with commonly used fluorophores and is suitable for multi-colour whole cell imaging. We have used 3D-SIM to explore the parasite's virulence protein export system and the structural basis of the remarkable shape-shifting that accompanies sexual development.

An alternative high resolution technique that can be performed on whole hydrated cells is transmission x-ray cryo-tomography. Due to the short wavelengths of x-rays this technique can achieve higher resolution than optical microscopy. We have undertaken tomographic imaging of the digestive system of the malaria parasite, using X-ray imaging in the “water window” to exploit the natural contrast of biological samples. The fact that the x-ray absorbance is proportional to the concentration of absorbing material permits a comparative analysis of the density of biological material in different cellular compartments.

Electron tomography is increasingly popular as a method to obtain 3D ultrastructural views of sections of biological material up to ~400 nm. Electron absorption and scattering, radiation damage and signal to noise issues hamper the analysis of thicker sections at intermediate voltage, however methods have been developed that permit tiling and stitching of serial tomograms. Immunoelectron tomography can be combined with serial sectioning techniques to permit high resolution imaging of whole cells. Using 3D-Electron Tomography we have undertaken ultrastructural analyses of whole *P. falciparum*-infected erythrocytes.